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ORIGINAL ARTICLE

Effects of tamoxifen on traumatic brain injury-induced depression in male rats

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tamoxifen

Abstract *Background/Introduction:* Previous studies have investigated the neuroprotective effects of tamoxifen (TMX), but its antidepressant-like effects in traumatic brain injury (TBI) remain unclear.
Purposes/Aims: The present study was conducted to determine whether TMX can attenuate TBI-induced depression-like behavior and whether this effect involves the activation of extracellular signal-regulated kinase 1/2 (ERK1/2).
Methods: Anesthetized male Sprague–Dawley rats were divided into four groups: sham-operated controls, TBI controls, TBI + TMX treatment (1 mg/kg), and TMX (1 mg/kg) + ERK1/2 antagonist, SL327 (30 mg/kg). Depression-like behaviors were evaluated through forced swim tests on Day 4, Day 8, and Day 15. On Day 15 after TBI, phosphorylated ERK1/2 (*p-ERK1/2*) expression was investigated by Western blotting; neuronal apoptosis, p-ERK1/2, B-cell chronic lymphocytic leukemia/lymphoma 2 (BCL2), and brain-derived neurotrophic factor (*BDNF*) expression in neuronal cells were evaluated using double immunofluorescence.
Results: On Day 15 after TBI, TMX significantly reduced the duration of TBI-induced immobility compared with the TBI controls. The frequency of neuronal apoptosis and numbers of BCL2-positive, BDNF-positive, and p-ERK1/2-positive neuronal cells in hippocampal CA3 were significantly improved by TMX. However, these TMX effects were significantly blocked by SL327 administration.
Conclusion: Our results suggest that intraperitoneal injection of TMX may ameliorate TBI-induced depression-like behavior in rats by increasing neuronal *p-ERK1/2* expression, which may be associated with neuronal *Bcl2* and *BDNF* expression and decreased neuronal apoptosis.

Conflicts of interest: All authors report no biomedical financial interest or potential conflict of interest.

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This effect might represent a mechanism underlying the recovery from depression-like behavior.

1. Introduction

Depression following a traumatic brain injury (TBI) is a severe complication. The incidence of posttraumatic depression is estimated to range from 10% to 77%, with variation according to the studied population, diagnostic criteria, and rating instruments used.[1] This post-TBI neuropsychiatric impairment contributes to disability after TBI and has become a chronic condition in an estimated 3.17 million Americans.[2] Therefore, developing novel therapeutic strategies and identifying new effective therapies are crucial.

The hippocampus is a crucial component of the limbic system, which is a site of the central nervous system that is involved in depressive behavior.[3] Previous studies have reported that hippocampal CA3 neurons appear to be particularly vulnerable to TBI,[4,5] which increases the likelihood that an insult to the hippocampus CA3 can induce hippocampal cell apoptosis and lead to depressive behavior.

The extracellular signal-regulated kinase (ERK1/2) pathways are associated with cell survival and apoptosis[6] and ERK1/2-mediated neuroprotective effects such as those induced through BCL2 and BDNF.[7,8] A previous study reported that the ERK1/2 signal transduction pathway in the hippocampus, but not that in the amygdala, may be involved in TBI-induced depression-like behavior in rats.[9] Intraperitoneal tamoxifen (TMX), a selective estrogen receptor modulator (SERM), may ameliorate TBI in rats by increasing neuronal ERK1/2 phosphorylation, which might lead to an increase in neuronal *Bcl2* expression and decrease in neuronal apoptosis after cortical ischemia.[10] Furthermore, Walf and Frye[11] demonstrated that subcutaneous or direct administration of estrogen into the hippocampus elicited antidepressant-like behavior compared with vehicle administration. TMX treatment in cultured hippocampal neurons increased the expression of the antiapoptotic protein, BCL2, an outcome linked to the neuroprotective effect of estrogen.[12] These phenomena increase the likelihood that TMX may have beneficial effects on TBI-induced apoptosis in hippocampal cells and subsequent antidepressant effects.

The neuroprotective effects of estrogen in TBI have been evaluated.[13] However, applying estrogen as a neuroprotective agent in humans may have several limitations such as causing estrogen-dependent peripheral tumors. In the current study, we selected TMX because, being a nonsteroidal SERM, its estrogen-like neuroprotective activity in the brain can act as an alternative agonist to estrogen[14]; it is brain—blood barrier permeable, achieving an increased concentration in the brain and serum upon administration[15]; and most importantly, the direct mechanism of TMX in TBI-induced depression-like behavior remains unclear.

The forced swim test (FST) is one of the most common animal models for assessing depression-like behavior. Longer periods of immobility during the test indicate more severe depression-like behaviors. Several antidepressants have been consistently shown to reduce the duration of immobility during testing by increasing active escape behaviors.[16,17]

In the current study, we hypothesized that TMX would have therapeutic effects on TBI-induced depression-like behaviors and that its beneficial effects may be associated with *ERK1/2* expression. To test this hypothesis, we applied SL327,[18] a brain-penetrating selective inhibitor of ERK1/2 that selectively inhibits phosphorylated ERK1/2 (p-ERK1/2), in the brain following systematic administration.[19] We investigated whether TMX in the presence or absence of SL327 activates neuronal ERK1/2, BCL2, and BDNF responses; reduces neuronal cell apoptosis; and ameliorates depression-like behaviors (evaluated by FST) after TBI in adult rats.

2. Methods

2.1. Animals

Adult male Sprague—Dawley rats weighing 397 ± 23 g were used in this study. The animals were maintained under a 12-hour light/12-hour dark cycle and allowed free access to both food and water. Before the study was initiated, all protocols for the study followed the Animal Protection Act, Council of Agriculture, Executive Yuan, Taiwan and were approved by the Chi-Mei Medical Center's Animal Care and Use Committee (IACUC; Tainan, Taiwan) for all experimental procedures (IACUC Approval No: 100120711). The protocols also conformed to the National Institutes of Health guidelines (Publication No. 85-23, revised 1985) including minimizing discomfort to animals during surgery and the recovery period.

2.2. TBI

The rats were anesthetized by intraperitoneal administration of a mixture of ketamine [44 mg/kg, intramuscularly (i.m.); Nankuang Pharmaceutical, Tainan, Taiwan], atropine (0.062633 mg/kg, i.m.; Sintong Chemical Ind. Co., Taoyuan, Taiwan), and xylazine (6.77 mg/kg, i.m.; Bayer, Leverkusen, Germany). A craniectomy (radius = 2 mm) 4 mm from the bregma and 3 mm from sagittal sutures in the right parietal cortex was performed using a stereotaxic frame. After craniectomy and implantation of an injury